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# Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis

Rie Watanabe-Fukunaga<sup>\*</sup>, Camilynn I. Brannan<sup>†</sup>, Neal G. Copeland<sup>†</sup>,  
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Fas antigen is a cell-surface protein that mediates apoptosis. It is expressed in various tissues including the thymus and has structural homology with a number of cell-surface receptors, including tumour necrosis factor receptor and nerve growth factor receptor. Mice carrying the lymphoproliferation (*lpr*) mutation have defects in the Fas antigen gene. The *lpr* mice develop lymphadenopathy and suffer from a systemic lupus erythematosus-like autoimmune disease, indicating an important role for Fas antigen in the negative selection of autoreactive T cells in the thymus.

T-CELL precursors arise in the bone marrow and then mature in the thymus after interaction with the thymic microenvironment<sup>1</sup>. During maturation, T cells recognizing self-antigens are destroyed by a process called apoptosis, whereas others are positively selected<sup>2,3</sup>. The lymphoproliferation mutation (*lpr*) seems to interfere with T-cell maturation. The *lpr* is autosomal recessive and the phenotype includes formation of multiple autoantibodies and accumulation of large numbers of non-malignant CD4<sup>+</sup>CD8<sup>+</sup> T lymphocytes in lymph nodes and the spleen<sup>4</sup>. Two independent spontaneous *lpr* mutations have been identified, *lpr* and *lpr*<sup>g</sup>. The original *lpr* mutation occurred during the derivation of the MRL/MpJ strain<sup>5</sup> and has been transferred onto a number of other inbred strain backgrounds, including C3H and C57BL<sup>6</sup>. The other mutation is *lpr*<sup>g</sup> in the CBA/KJms mouse strain<sup>7</sup>. The clinical syndrome of *lpr* and *lpr*<sup>g</sup> mice is characterized by hypergammaglobulinaemia, anti-DNA antibodies, rheumatoid factor, and circulating immune complexes as well as arthritis and glomerulonephritis, which resembles human systemic lupus erythematosus (SLE)<sup>8</sup>. Studies of *lpr* mice suggest that there is a defect in negative selection of self-reactive T lymphocytes in the thymus<sup>9,10</sup>. Excessive numbers of self-reactive T lymphocytes released into peripheral organs seem to be responsible for the autoimmune disease in *lpr* mice.

Here we provide evidence that *lpr* encodes the structural gene

for the mouse Fas antigen<sup>10</sup>. A mouse monoclonal antibody has been prepared which has a cytolytic activity on human cells expressing the Fas antigen<sup>11</sup>. Cloning of Fas antigen complementary DNA from human<sup>12</sup> and mouse cells<sup>10</sup> indicates that the Fas antigen is a protein containing a single transmembrane domain with a calculated *M<sub>r</sub>* of 35,000 (35 K) (refs 10, 12). Fas antigen from both species shows structural homology with a number of cell-surface receptors, including tumour necrosis factor (TNF) receptors and the low-affinity nerve growth factor receptor<sup>10,12</sup>. Northern analysis indicates the Fas antigen messenger RNA is expressed in a limited number of tissues, including the thymus, liver, ovary and heart<sup>10</sup>. When human Fas antigen is expressed in mouse cell lines, it can induce Fas antigen antibody-triggered cell death<sup>12</sup>. Characterization of the process of cell death indicates that Fas antigen mediates apoptosis<sup>12</sup>. Expression of Fas antigen in the thymus and its role in apoptosis provides an explanation for the phenotypes of *lpr* mice. These studies further suggest a role for the Fas antigen in the negative selection of autoreactive T cells in the thymus.

## Expression of Fas mRNA in *lpr* mice

The mouse Fas antigen gene has been assigned to chromosome 19 by interspecific backcross analysis<sup>10</sup>. When our linkage map of mouse chromosome 19 was aligned with a composite linkage map (GBASE, Jackson Laboratory) we found that the Fas antigen locus mapped near *lpr*<sup>13</sup>.

To determine whether the *lpr* mutation affects Fas antigen mRNA expression, northern analysis of wild-type and mutant *lpr* tissues was done using a mouse Fas antigen cDNA probe. Total cellular RNAs were isolated from two different strains carrying the *lpr* mutation (MRL *lpr/lpr* and C3H *lpr/lpr*) and their parental wild-type controls (MRL +/+ and C3H +/+) and in agreement with previous observations<sup>10</sup>, a 2.1 kilobase (kb) Fas antigen mRNA is detected in the liver and thymus of mice wild type at *lpr* (Fig. 1a). Almost no Fas mRNA is observed in homozygous *lpr* mice. Reprobing the same blot with human elongation factor-1 $\alpha$  (EF-1 $\alpha$ ) cDNA<sup>14</sup> reveals a band of 2.2 kb in all RNA preparations (Fig. 1b). To confirm the absence of Fas mRNA in *lpr* mice, single-stranded cDNAs were synthesized from thymus RNA of wild-type and *lpr* mice and amplified by the polymerase chain reaction (PCR), using Fas antigen oligonucleotide primers. PCR amplification of RNA from wild-type mice gave a band of the expected size (420 bp), whereas no such band was amplified from *lpr* mice (Fig. 1c).

<sup>†</sup> To whom correspondence should be addressed.

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for the mouse Fas antigen<sup>10</sup>. A mouse monoclonal antibody has been prepared which has a cytolytic activity on human cells expressing the Fas antigen<sup>11</sup>. Cloning of Fas antigen complementary DNA from human<sup>12</sup> and mouse cells<sup>10</sup> indicates that the Fas antigen is a protein containing a single transmembrane domain with a calculated *M<sub>r</sub>* of 35,000 (35 K) (refs 10, 12). Fas antigen from both species shows structural homology with a number of cell-surface receptors, including tumour necrosis factor (TNF) receptors and the low-affinity nerve growth factor receptor<sup>10,12</sup>. Northern analysis indicates the Fas antigen messenger RNA is expressed in a limited number of tissues, including the thymus, liver, ovary and heart<sup>10</sup>. When human Fas antigen is expressed in mouse cell lines, it can induce Fas antigen antibody-triggered cell death<sup>12</sup>. Characterization of the process of cell death indicates that Fas antigen mediates apoptosis<sup>12</sup>. Expression of Fas antigen in the thymus and its role in apoptosis provides an explanation for the phenotypes of *lpr* mice. These studies further suggest a role for the Fas antigen in the negative selection of autoreactive T cells in the thymus.

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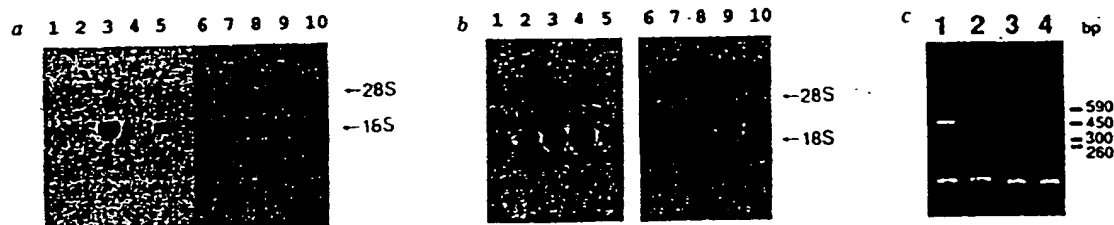


FIG. 1 Little expression of Fas antigen mRNA in mice homozygous for the *lpr* mutation. Northern blot analysis of total cellular RNA from various mouse strains hybridized with mouse Fas antigen cDNA<sup>10</sup> (a) or human EF-1α cDNA<sup>14</sup> (b). Total cellular RNAs (9 μg) are from the liver (lanes 1, 2, 6, 7) and thymus (lanes 3, 4, 8, 9) of MRL/MpJ +/+ (lanes 1, 3), MRL/MpJ *lpr/lpr* (lanes 2, 4), C3H/HeJ +/+ (lanes 6, 8), and C3H/HeJ *lpr/lpr* (lanes 7, 9) mice. Total cellular RNA (4.5 μg) from mouse L929 cells was also analysed (lanes 5, 10). Positions of 18S and 28S ribosomal RNAs are indicated. c. The Fas antigen mRNA detected by reverse PCR. Single-stranded cDNA was synthesized using total cellular RNAs from the thymus of MRL/MpJ +/+ (lane 1), MRL/MpJ *lpr/lpr* (lane 2), C3H/HeJ +/+ (lane 3) and C3H/HeJ *lpr/lpr* (lane 4) mice, and Fas antigen cDNA was amplified by PCR. Size markers (base pairs) are shown on the right. METHODS. Total cellular RNAs, prepared by the guanidine isothiocyanate/acid phenol method<sup>29</sup>, were denatured at 65 °C for 5 min in 2.2 M formaldehyde

and 50% deionized formamide, and electrophoresed through 1.5% agarose gels containing 2.2 M formaldehyde<sup>30</sup>. RNA was transferred to a nitrocellulose filter and hybridized with [<sup>32</sup>P] labelled probe DNA. The probe DNAs used are a 1.5-kb *Eco*RI fragment containing mouse Fas antigen cDNA<sup>10</sup>, or a 1.8-kb *Bam*HI fragment containing human EF-1α cDNA<sup>14</sup>. The reverse PCR was done as described<sup>23</sup>. Random hexamer-primed single-stranded cDNA was synthesized in a final volume of 50 μl with 80 units avian myeloblastosis reverse transcriptase and 2 μg total RNA. An aliquot (5 μl) of the reaction mixture was diluted with 50 μl of the PCR buffer<sup>22</sup>, and the PCR reaction was done using 2.5 units *Thermus aquaticus* DNA polymerase (*Taq* polymerase). The conditions for the PCR were 1.5 min at 95 °C, 1.5 min at 60 °C, and 3 min at 72 °C, 30 cycles. The primers spanned nucleotides 45–64 and nucleotides 448–467 in the coding sequence of mouse Fas antigen cDNA<sup>10</sup>.

### Rearrangement of the Fas antigen gene

To determine whether the absence of Fas antigen mRNA in *lpr* mice is associated with a rearrangement of the Fas antigen gene, Southern analysis of genomic DNAs from mutant *lpr* and wild-type mice was done using a full-length Fas antigen cDNA probe. Several bands were observed in *Eco*RI-, *Bam*HI- and *Pst*I-digested genomic DNA from wild-type MRL and C3H mice (Fig. 2a, b). Except for an extra 9-kb band observed in *Bam*HI-digested DNA from C3H mice (Fig. 2b, lane 1), all bands were identical in size between the two strains. The genomic locus for the wild-type mouse Fas antigen gene has been isolated (R.W.F. and S.N., unpublished observations). A comparison of the restriction map of the cloned genomic locus with DNA fragments observed in Southern analysis indicates that the Fas antigen gene is present in a single copy per haploid mouse genome and that some bands detected by Southern analysis represent closely migrating doublets.

Southern analysis of DNA from MRL *lpr/lpr* mice showed extra bands of 8 kb or 9 kb in *Bam*HI- or *Eco*RI-digested DNAs, respectively (Fig. 2a, lanes 3, 6). The 5-kb *Pst*I fragment in wild-type DNA (Fig. 2a, lane 7) was replaced by a slightly larger fragment (Fig. 2a, lane 9). When DNAs from wild-type and mutant strains were mixed before restriction enzyme digestion, bands expected for both wild-type and mutant strains were

observed (Fig. 2a, lanes 2, 5, 8), indicating that the extra bands seen in *lpr* DNA are not due to artefacts generated during restriction enzyme digestion. Southern analysis of DNA from C3H *lpr/lpr* mice gave bands identical to those in MRL *lpr/lpr* mice (Fig. 2b). These results indicate that the structural gene for the Fas antigen is rearranged in *lpr* mice.

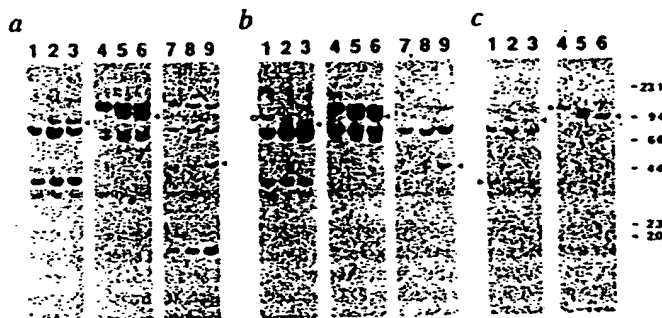
To determine which region of the Fas antigen gene was rearranged, mouse Fas antigen cDNA was divided into four parts and each part was used as a probe for Southern analysis of wild-type and *lpr* DNAs. Rearrangement of the Fas antigen gene was detected only with the probe that contains nucleotides 115–580 of mouse Fas antigen cDNA<sup>10</sup>. The 4-kb *Bam*HI and 10-kb *Eco*RI DNA fragments seen in wild-type DNA were replaced by 8-kb and 9-kb fragments, respectively, in *lpr* DNA (Fig. 2c). Preliminary analysis of Fas antigen genomic DNA clones suggests that the 4-kb *Bam*HI fragment detected in wild-type DNA contains exon 3 and that the rearrangement seen in *lpr* mice has occurred in intron 2 (R.W.F. and S.N., unpublished observations).

### A point mutation in Fas antigen

To confirm that Fas antigen is the structural gene for *lpr*, a second *lpr* mutation, *lpr*<sup>ca</sup>, was analysed. Northern analysis of RNAs from liver and thymus of CBA *lpr*<sup>ca</sup>/*lpr*<sup>ca</sup> mice indicates

FIG. 2 Southern analysis of the Fas antigen gene in various strains. High *M*<sub>r</sub> DNA was prepared from the spleen of a, c, MRL/MpJ and b, C3H/HeJ mice. Genomic DNA (10 μg per lane) from wild-type (lanes 1, 4, 7) and *lpr/lpr* mutant mice (lanes 3, 6, 9), and their 1:1 mixture (lanes 2, 5, 8) was digested with *Bam*HI (lanes 1–3), *Eco*RI (lanes 4–6) or *Pst*I (lanes 7–9). Southern analysis was done using a full-length mouse Fas antigen cDNA<sup>10</sup> (a, b) or coding sequences between nucleotides 115–580 (c) as probes. Filled arrowheads, DNA fragments rearranged in *lpr* mice; open arrowheads, the corresponding DNA fragments in wild-type mice; Open circle, the polymorphic DNA fragment observed in *Bam*HI-digested C3H DNA.

METHODS. High *M*<sub>r</sub> chromosomal DNA, prepared from the spleen<sup>30</sup>, was digested with various restriction enzymes, electrophoresed on a 0.8% agarose gel, and transferred to a nylon membrane under alkaline conditions as described<sup>30</sup>. Probe DNA fragments were prepared by PCR using mouse Fas antigen cDNA<sup>10</sup> as a template, and labelled



with <sup>32</sup>P by the random primer labelling method (Boehringer). Hybridization was carried out under high stringency<sup>30</sup>. Sizes of marker DNA are shown in kb on the right.

that this mutant mouse expresses Fas antigen mRNA to the same extent as wild-type CBA  $+/+$  mice (Fig. 3a). After reverse transcription of CBA  $+/+$  RNA, the coding sequence of the Fas antigen cDNA was amplified by PCR and inserted into pBluescript. The PCR product has a sequence identical to that of mouse Fas antigen cDNA isolated from the mouse BAM3 cell line<sup>10</sup> (derived from BALB/c mice), except at nucleotide positions 162, 163, 283, 379 and 505. These differences probably represent polymorphisms between BALB/c and CBA mice. The sequence of three independent Fas antigen cDNA clones, obtained with RNA from CBA  $lpr^{cr}/lpr^{cr}$  mice, all show a transition of T to A at nucleotide 786, in addition to the polymorphic mutations mentioned above. This mutation causes the replacement of an asparagine for an isoleucine in the cytoplasmic region of the Fas antigen (Fig. 3b), which is highly conserved between the Fas antigen and TNF receptor type I.

To establish whether this mutation abolishes the ability of the Fas antigen to transduce the apoptotic signal, and to overcome the lack of antibodies against the mouse Fas antigen, two chimaeric Fas antigen molecules were constructed from human and mouse cDNAs. Both chimaeras carry the extracellular and transmembrane domains of the human Fas antigen cDNA. The cytoplasmic domains are derived from Fas antigen cDNA of wild-type or  $lpr^{cr}$  mice, respectively. The chimaeric cDNAs were expressed in mouse L929 cells, and the susceptibility of the transformants to the Fas antibody-induced apoptosis was assayed<sup>12</sup>.

Transformants expressing the chimaera with the wild-type Fas antigen cytoplasmic tail were effectively killed by the Fas antibody (Fig. 4), indicating that the cytoplasmic region of the wild-type mouse Fas antigen can transduce the apoptotic signal into cells. Cells expressing the chimaera with the mutated mouse Fas antigen from  $lpr^{cr}$  mice were resistant to the cytolytic activity of the anti-human Fas antibody. These results indicate that the Fas antigen encoded by  $lpr^{cr}$  mutant mice is unable to transduce the apoptotic signal into cells, and that  $lpr$  encodes Fas antigen.

### Discussion

We have shown that  $lpr$  mice carry defects in the Fas antigen which mediates apoptosis<sup>12</sup>. Bone marrow transplantation studies indicate that the  $lpr$  mutation causes an intrinsic T-

lymphocyte abnormality responsible for lymphadenopathy and autoantibody production<sup>15</sup>. The Fas antigen has been expressed in the thymus<sup>10</sup> and on activated or transformed T cells<sup>12</sup>. Thus it is possible that precursor T cells reacting with self-antigens express the Fas antigen during their thymic development and are killed by interaction with stroma cells in the thymus. No functional Fas antigen is expressed in  $lpr$  mice. Therefore, autoreactive  $lpr$  T cells could escape thymic selection, resulting in the lymphadenopathy and autoimmune disease in  $lpr$  mice<sup>4,15</sup>.

Mice carrying the generalized lymphoproliferative disease mutation (*gld*) show a clinical syndrome which is indistinguishable from that of  $lpr$  mice<sup>6,16</sup>. Like  $lpr$ , *gld* is autosomal recessive but *gld* maps to mouse chromosome 1<sup>17</sup>. Bone marrow transplantation<sup>18</sup> indicates that these two mutations are expressed by different cell compartments and affect an interacting pair of molecules, suggesting that the structural gene for *gld* may encode the ligand for Fas antigen. The molecule affected by the *gld* mutation is thought to be expressed in bone marrow-derived cells<sup>18</sup>. That the thymic stromal cells responsible for T-cell depletion are derived from the bone marrow<sup>1,19,20</sup> supports this.

Transgenic mice expressing a rearranged T-cell receptor for the H-Y male antigen and carrying the  $lpr$  mutation have been constructed<sup>9,21</sup>. Although male  $lpr$  mice carrying the transgene show an increased number of autoreactive T-cells in the spleen and an enhanced production of autoantibodies<sup>9</sup> relative to female mice, clonal deletion of autoreactive T-cells was still observed. These results indicate that in addition to the Fas antigen, other molecules have a role in the clonal deletion of autoreactive T-cells, although it is not possible to rule out the weak expression of the Fas antigen in  $lpr$  mice. The involvement of defective B-cells in the autoimmune disease of  $lpr$  mice has also been suggested<sup>22,23</sup>. Because Fas antigen can be expressed in activated B lymphocytes<sup>11</sup>, B-cell producing autoantibodies could survive longer in  $lpr$  mice than normal mice. Such an extension of the functional lifespan of B lymphocytes could cause autoimmune disease in a similar way to that proposed for transgenic mice carrying an unregulated *bcl-2* gene<sup>24</sup>.

Transfer of bone marrow stem cells from  $lpr$  mice into irradiated wild-type mice causes a severe graft-versus-host-like disease (GVHD)<sup>25</sup>. But this is not seen when bone marrow stem cells from  $lpr^{cr}$  mice are transferred into irradiated wild-type

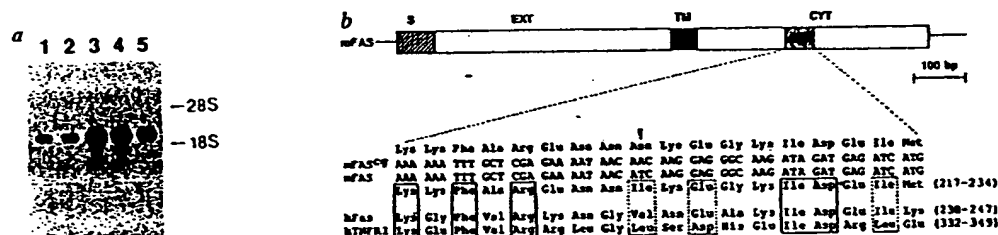
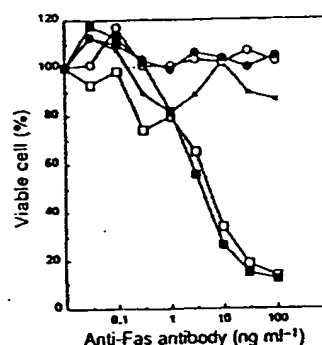


FIG. 3 Structure of the Fas antigen gene in mice homozygous for the  $lpr^{cr}$  mutation. a Northern analysis of Fas antigen mRNA in  $lpr^{cr}$  mice. CBA/K1.Jms  $+/+$  and CBA/K1.Jms  $lpr^{cr}/lpr^{cr}$  mice total cellular RNA (9  $\mu$ g) prepared from the liver (lanes 1, 2) or thymus (lanes 3, 4) of wild-type (lanes 1, 3) or mutant (lanes 2, 4) mice was analysed using mouse Fas antigen cDNA as probe. Total RNA from L929 cells (lane 5) was also analysed. Positions of 18S and 28S rRNA are indicated. b Point mutation in the cytoplasmic region of the Fas antigen gene of  $lpr^{cr}/lpr^{cr}$  mice. The coding region of the Fas antigen gene expressed in the thymus and liver of wild-type and mutant mice was amplified by PCR, and the nucleotide sequence determined after insertion into pBluescript. Upper panel, Predicted structure of the mouse Fas antigen protein. S, signal sequence; EXT, extracellular region; TM, transmembrane region; CYT, cytoplasmic region. Lower panel, Nucleotide sequence and deduced amino-acid sequence of the Fas antigen cDNA of  $lpr^{cr}$  (mFas<sup>cr</sup>) and wild-type (mFas) mice at the site of the mutation. Amino-acid sequences of the corresponding region of the human Fas antigen (hFas)<sup>22</sup> and human TNF type I receptor (hTNFR)<sup>22</sup> are included. Sets of three identical amino-acid

residues at one aligned position are boxed by solid lines, sets of three residues regarded as favoured substitutions are boxed by dotted lines. The arrowhead indicates the position of the mutation in the Fas antigen of  $lpr^{cr}$  mice.

**METHODS.** Total RNA (2  $\mu$ g) from the liver or thymus of CBA  $+/+$  or CBA  $lpr^{cr}/lpr^{cr}$  mice was used as a template for single-stranded cDNA synthesis. PCR was carried out as described<sup>21</sup>. The oligonucleotides used as primers are GGAATCGCGCTGTTTCCTTCTGCTCA (5' primer) and GGTCAGCAGGAGTGGCAATGTCAAT (3' primer), which contains the sequence in the 5' or 3' non-coding region of the mouse Fas antigen cDNA<sup>10</sup>, respectively. PCR conditions were 1.5 min at 95 °C, 1.5 min at 60 °C, and 3 min at 72 °C, 50 cycles. The amplified DNA fragment was digested with EcoRI and SalI, and inserted into pBluescript KS(+). The sequencing reaction was done by the dideoxynucleotide chain-termination method using T7-DNA polymerase (Pharmacia) and [ $\alpha$ -<sup>32</sup>S]dATP (Amersham). As primers for the sequencing reaction, a set of 20-base oligonucleotides specific for the coding region of the mouse Fas antigen gene was used.

FIG. 4 Inability of the Fas antigen encoded by *lpr*<sup>cr</sup> mice to mediate apoptosis. Mouse L929 cell transformed by and expressing a chimeric human-mouse Fas antigen containing the wild-type mouse Fas antigen (□, ■) or the mutated mouse Fas antigen (○, ●) cytoplasmic region were incubated at 37 °C for 6 h with various concentrations (0.03–100 ng ml<sup>-1</sup>) of anti-human Fas antibody in the presence of 0.5 μg ml<sup>-1</sup> actinomycin D. After incubation, viable cells were stained with crystal violet, and dye uptake was quantified by measuring absorbance at 540 nm using an automated Micro-ELISA autoreader. The parental L929 cells (x) were also treated with anti-human Fas antibody in the presence of actinomycin D. Assays were done in duplicate for two independent cell lines transformed by and expressing each chimeric Fas antigen, and the average values for each transformant were plotted.



**METHODS.** A hybrid cDNA between mouse and human Fas antigen cDNAs was constructed by the recombinant PCR method<sup>32</sup>. The primary PCR was done with two sets of primers. A part of the human Fas antigen cDNA was amplified with a forward primer (primer A) containing human sequence from 696–715<sup>12</sup>, and a 41-base hybrid primer (primer B) containing the sequence complementary to mouse Fas antigen cDNA from 586–608<sup>10</sup>, and to human Fas antigen cDNA from 725–743<sup>12</sup>. A part of mouse Fas antigen cDNA was amplified using the wild-type mouse Fas antigen cDNA or the mutated cDNA from *lpr*<sup>cr</sup> mice as template. The oligonucleotide complementary to primer B, and a 20-base oligonucleotide (primer C) complementary to the sequence from 984–1,003 of the mouse Fas antigen cDNA<sup>10</sup> were used as primers. The conditions for the PCR were 1 min at 94 °C, 2 min at 55 °C and 2 min at 72 °C, 15 cycles. Products were isolated by agarose gel electrophoresis, and treated with T4 DNA polymerase to flush the ends. The two DNA fragments obtained by the primary PCRs were then mixed at 1:1, and the secondary PCR was done for 20 cycles as above using primers A and C. The product was digested with *Bam*HI and *Kpn*I, and the 330-bp DNA

fragment was isolated. This fragment was ligated with the 700-bp *Xba*I-*Bam*HI fragment containing the 5' part of human Fas antigen cDNA<sup>12</sup> and the 660-bp *Kpn*I-*Xba*I fragment containing the 3' part of mouse Fas antigen cDNA<sup>10</sup>, and introduced into mammalian expression vector pEF-BOS<sup>34</sup>. All constructions were confirmed by DNA sequencing. The expression plasmid for the human-mouse chimeric Fas antigen was introduced into mouse L929 cells<sup>12</sup>. After 9 days, individual G-418-resistant colonies were isolated, and clones transformed by and expressing the chimeric Fas antigen were identified by FACS analysis using mouse anti-human Fas monoclonal antibody.

mice<sup>24</sup>. These two observations can be explained as follows. Fas antigen mRNA is not expressed in *lpr* mice. When *lpr* stem cells introduced in wild-type mice become mature T-cells, they recognize the Fas antigen expressed in the host cells as a foreign antigen or 'non-self' and induce the GVHD syndrome. Mice carrying the *lpr*<sup>cr</sup> allele express the Fas antigen, although in a non-functional form. Therefore, mature T-cells derived from stem cells of *lpr*<sup>cr</sup> mice can recognize host cells expressing the normal Fas antigen as 'self'.

Our results explain the phenotype observed in double heterozygous *lpr*<sup>cr</sup>/*gld*<sup>+</sup>, and *lpr*<sup>cr</sup>/*gld*<sup>+</sup> mice<sup>7</sup>. Although the original *lpr* mutation fails to complement *gld* in double heterozygotes, *lpr*<sup>cr</sup> can complement *gld*<sup>+</sup>; *lpr*<sup>cr</sup>/*gld*<sup>+</sup> double heterozygotes develop a disease similar to that observed in *lpr*/*lpr* or *gld*/*gld* mice, although it is less pronounced. These phenotypic differences can be explained by the nature of the Fas antigen lesions found in *lpr* and *lpr*<sup>cr</sup> mice. In *lpr* mice, Fas antigen is not expressed and can thus not bind ligand. In *lpr*<sup>cr</sup> mice, a non-functional receptor is expressed that can still presumably bind ligand. In *lpr*<sup>cr</sup>/*gld*<sup>+</sup> double heterozygotes,

the non-functional receptor could compete for ligand, further lowering the effective concentration of ligand below that normally found in *gld*<sup>+</sup> mice. This reduced concentration of ligand could produce a phenotype similar to that seen in *lpr*/*lpr* or *gld*/*gld* mice.

Fas antigen is expressed in the thymus and in the liver, heart and ovary<sup>10</sup>. Abnormalities of these tissues have not been described in *lpr* mice, although CD4<sup>+</sup> CD8<sup>+</sup> T-lymphocytes which accumulate in *lpr* mice seem to proliferate in the liver<sup>27</sup>. Because the liver can support extrathymic development of T-lymphocytes if the thymus is inactive or absent<sup>27</sup>, it is possible that cells expressing Fas antigen in the liver are precursor cells for T-lymphocytes.

We have provided evidence that the *lpr* locus encodes Fas antigen. This finding should help to elucidate the process regulating normal T-cell maturation in the thymus, and the mechanism underlying formation of autoimmune disease in mice. Finally, human patients displaying a similar phenotype as *lpr* mice have been identified<sup>28</sup> and it will be interesting to examine the structure of the Fas antigen gene in these patients. □

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